SCN Outputs and the Hypothalamic Balance of Life

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Abstract The circadian clock in the suprachiasmatic nucleus (SCN) is composed of thousands of oscillator neurons, each dependent on the cell-autonomous action of a defined set of circadian clock genes. Still, the major question remains how these individual oscillators are organized into a biological clock producing a coherent output able to time all the different daily changes in behavior and physiology. In the present review, the authors discuss the anatomical connections and neurotransmitters used by the SCN to control the daily rhythms in hormone release. The efferent SCN projections mainly target neurons in the medial hypothalamus surrounding the SCN. The activity of these preautonomic and neuroendocrine target neurons is controlled by differentially timed waves of, among others, vasopressin, GABA, and glutamate release from SCN terminals. Together, the data on the SCN control of neuroendocrine rhythms provide clear evidence not only that the SCN consists of phenotypically (i.e., according to neurotransmitter content) different subpopulations of neurons but also that subpopulations should be distinguished (within phenotypically similar groups of neurons) based on the acrophase of their (electrical) activity. Moreover, the specialization of the SCN may go as far as a single body structure, that is, the SCN seems to contain neurons that specifically target the liver, pineal, and adrenal.

Key words GABA, glutamate, vasopressin, melatonin, glucose, corticosterone

The periodic succession of night and day has influenced life on earth for millions of years. In mammals, these periodic changes in the environment have been “internalized” in the form of an endogenous circadian clock. Its main function is to organize the time course of physiological, hormonal, and behavioral processes to enable the organism to anticipate these changing environmental conditions properly. Circadian rhythms serve to temporally partition the organism’s ecological niche and enable it to anticipate and adapt optimally to ambient conditions. Although it is thought that this ability to anticipate environmental changes imparts survival advantages to an organism, an important aspect of circadian control may also be
to time and synchronize metabolic processes within the organism, that is, to optimize metabolic networks by enabling a temporal partitioning of metabolic events within and between different tissues, for example, by temporally separating chemically antagonistic reactions and by limiting the expression of certain enzymes to the time of day they are needed (Stratmann and Schibler, 2006 [this issue]).

In mammals, the daily rhythms in behavior and physiology are generated and orchestrated from within a biological or circadian clock located in the anterior hypothalamus. The location of the responsible clock within the hypothalamic suprachiasmatic nucleus (SCN) was discovered in the early 1970s (Moore and Eichler, 1972; Stephan and Zucker, 1972). Conclusive evidence that the SCN indeed comprises the master circadian pacemaker came from combined lesion and transplantation studies. Transplantation of SCN tissue from mutant donor animals into SCN-lesioned wild-type hosts (or vice versa) conferred the circadian phenotype of the donor to the host (Ralph et al., 1990; Sujino et al., 2003). More recently, it became clear that the endogenous rhythm of the master oscillator is generated by a suite of clock genes, forming different sets of interlocking transcriptional-translational feedback loops (see the special JBR issue on The Molecular Basis of Circadian Rhythms, October 2004, 19[5]). Despite this vast increase in recent knowledge, the link between the transcriptional and translational events of the molecular clock, on one hand, and the metabolic and electric activity of the SCN neurons (i.e., the output of the endogenous clock), on the other, is still not known. Moreover, the manner in which individual SCN neurons are assembled to create an integrated tissue pacemaker that can govern the circadian behavior of the whole animal is still unknown (Kuhlman and McMahon, 2006 [this issue]).

Clearly, the most obvious result of SCN output is the behavioral sleep/wake or rest/activity cycle (Stephan and Zucker, 1972). Nevertheless, hormone rhythms were also at the basis of the discovery of the SCN. In 1972, Moore and Eichler used the daily rhythm in rat adrenal corticosterone content as their readout for deciding whether they had effectively removed the long sought after circadian oscillator. Two years later, Moore and Klein (1974) used the rate-limiting enzyme for the rhythmic release of pineal melatonin, that is, N-acetyltransferase, to make a start with deciphering the SCN output pathways. From the early onset of (mammalian) chronobiology onward, an important question has been whether physiological processes such as the daily rhythms in energy metabolism, body temperature, and hormone release are gated by the behavioral sleep/wake and feeding/fasting rhythms or subject to an independent control by the circadian oscillator. Indeed, because in many species the temporal distribution of sleep and wakefulness is so clearly affected by the biological clock, it has been assumed that the daily rhythms of physiological events such as body temperature, blood pressure, and circulating concentrations of (metabolic) hormones are mainly induced by the behavioral rhythm, instead of being subject to a direct control of the biological clock. But as we will show in the present review, and in accordance with the proposed anticipatory role of the SCN, a direct SCN control of these physiological processes is clearly present.

SCN OUTPUTS AND BEHAVIOR

The 1st SCN transmitter to be demonstrated was vasopressin (VP) (Swaab et al., 1975), although at that time its function as a neurotransmitter was not yet recognized. Due to its pronounced day/night rhythm in CSF (Reppert et al., 1981), VP was characterized as a humoral output of the SCN, and to date, it is still the only SCN output that has been demonstrated to be secreted in a circadian rhythm in vivo. Clearly the daily fluctuations of VP in the CSF are a result of the day/night rhythm in the firing rate of VP-containing SCN neurons (Fig. 1), but it is still not clear whether VP in the CSF really acts as a humoral factor, or if it is merely spillover, that is, VP released as a neurotransmitter that is removed by diffusion. Infusion of a VP antagonist in the ventricular system, however, did not abolish the daily sleep/wake rhythm (Kruisbrink et al., 1987), which seems to argue against a neurotransmitter role of CSF VP in the sleep/wake rhythm. Despite its early discovery, the interest in VP as an important clock output rapidly disappeared when no gross abnormalities could be detected in the circadian rhythms of the Brattleboro rat, which bears a naturally occurring missense mutation in the gene encoding for VP (Groblewski et al., 1981). However, more recent observations renewed the interest in VP as an important clock-controlled output gene (Jin et al., 1999; Tousson and Meissl, 2004). The demonstration that transplanted SCN tissue surrounded by semipermeable membrane can still restore a circadian rhythm in locomotor activity showed that diffusible factors can emanate from the transplanted SCN (Silver et al., 1996).
Subsequently, factors such as TNF-α, prokineticin-2, and cardiotrophin-like cytokine have been proposed as humoral outputs of the circadian pacemaker in mammals that inhibit locomotor activity (Kramer et al., 2001; Cheng et al., 2002; Kraves and Weitz, 2006). In addition, neuromedin S has been proposed as an anorexigenic SCN signal (Mori et al., 2005). So far, no SCN factors promoting arousal or feeding activity have been proposed, but the elegant temporal chimera experiments of Vogelbaum and Menaker (1992) clearly indicate that, at least for locomotor activity, both stimulatory and inhibitory inputs from the SCN are to be expected. Moreover, in a recent review, it was concluded that the SCN actively promotes both wake and sleep at different phases of the 24-h L/D cycle, instead of only actively promoting wakefulness and passively gating sleep (Mistlberger, 2005). Together, these results suggest that there is a mélange of secreted SCN factors able to alternate between inhibition and stimulation of different behavioral outputs.

SCN OUTPUTS AND HORMONES

Transplantation experiments also unequivocally demonstrated that apart from humoral SCN factors, intact neural projections from the SCN to specific regional targets are needed for a complete restoration of function because transplants that successfully restored circadian locomotor activity failed to restore neuroendocrine rhythms (Lehman et al., 1987; Meyer-Bernstein et al., 1999). Furthermore, the experiments of Guo et al. (2005), using parabiosis between intact and SCN-lesioned animals, nicely demonstrated that nonneuronal mechanisms are not sufficient to reinstate circadian rhythms in all peripheral organs. Information on the distribution of SCN projections was initially obtained from neuroanatomical studies using tracing, immunocytochemistry, SCN lesions, or a combination of these methods (Hoorneman and Buijs, 1982; Watts et al., 1987). All these studies showed that the outflow of SCN information only pertained to the medial hypothalamus because most of the SCN projections were directed toward target areas that contained mainly interneurons, such as the MPOA, DMH, and subPVN. Despite their relative scarcity, direct connections to CRH-, TRH-, TH-, and GnRH-containing neurons were described as well (Kalsbeek and Buijs, 2002). More recently, an elegant experiment by De La Iglesia et al. (2003) provided functional evidence for the necessity of point-to-point neural connections to sustain neuroendocrine rhythms. Using behaviorally “split” female rats, they showed not only an antiphase of clock gene cycling in the left and right SCN but also a pronounced left/right asymmetry in activated LHrH neurons, as indicated by c-Fos expression. Thus, the previously reported circa 12-h LH secretory surges in behaviorally split animals are the result of alternating left- and right-sided stimulation of LHrH neurons, by point-to-point axonal SCN projections.

The 2 neuroendocrine rhythms that had been so important in revealing the oscillatory capacities of the SCN in the early 1970s (i.e., corticosterone and melatonin) also proved helpful in making a functional link between SCN output and circadian control. The 1st SCN transmitter to be clearly linked to a neuroendocrine rhythm was the VP-containing projection to the PVN/DMH area and its control of the daily rhythm of corticosterone release from the adrenal cortex (the melatonin rhythm will be discussed in the next paragraph). The prominent rhythm of VP release from SCN terminals (Fig. 1) and the proximity of these terminals to the CRH-containing endocrine neurons in the PVN led us to start investigating the functional significance of this anatomical pathway. Our initial physiology experiments in rats showed that the daily rhythm of corticosterone release was controlled by at least 2 SCN transmitters, that is, VP release within the DMH, which inhibits the activity of the HPA axis during the early part
of the light period, and an as yet unknown SCN transmitter that stimulates the activity of the HPA axis from the middle of the light period until the onset of the dark period (Kalsbeek and Buijs, 2002; Buijs and Kalsbeek, 2001). Because of their ACTH- and corticosterone-stimulating properties, VIP and GRP were initially proposed as possible stimulatory SCN transmitters (Kalsbeek et al., 1996). More recently, neuromedin U appeared as a possible candidate for this stimulatory SCN transmitter (Graham et al., 2005). However, timed antagonist studies are still lacking for the final functional prove. Moreover, none of the daily gene expression rhythms completely fits the expected profile. In addition to this neuroendocrine route, the SCN also proved to be connected to the adrenal via a polysynaptic projection involving the preautonomic neurons in the PVN and the spinal cord. This separate pathway enables the SCN to influence the sensitivity of the adrenal cortex to ACTH (Buijs et al., 1999; Ishida et al., 2005; Ulrich-Lai et al., 2006).

SCN OUTPUTS AND NERVES

Hormones present themselves broadly throughout the body and obtain their specificity by acting on their receptors expressed in specific tissues, whereas neurons deliver their message to a precisely targeted tissue in the body. The central clock also uses these 2 options to impose its rhythmicity onto the rest of the body. In fact, the circadian control of the daily corticosterone rhythm nicely shows how the SCN uses both of them to provide an optimum secretion of corticosterone, that is, a direct input to the neuroendocrine neurons to control the release of ACTH and the control of the adrenal sensitivity for ACTH via the autonomic nervous system.

This dual control mechanism may hold not only for endocrine glands but also for other organs (Fig. 2). For instance, at the time of awakening, the SCN increases not only insulin sensitivity to cause a physiologically relevant increase in (muscle) glucose uptake, but also simultaneously, glucose production by the liver to ensure sufficient glucose availability (Buijs and Kalsbeek, 2001).

The 1st SCN output pathway to be clearly linked to a neuroendocrine rhythm was the one that uses the sympathetic innervation of the pineal gland to connect the rhythmic activity of the SCN with the rhythmic release of melatonin. The major anatomical details of this neural pathway were revealed soon after the SCN was identified as the central pacemaker in mammals (Moore and Klein, 1974), but it was only with the help of transneuronal viral tracer injections in the pineal gland that the multisynaptic nature of the connection between the SCN, paraventricular nucleus (PVN) of the hypothalamus, preganglionic sympathetic neurons in the spinal cord, and the noradrenalin-containing neurons in the superior cervical ganglion could finally be confirmed. Manipulation of the GABAergic transmission at the level of the PVN proved to be very effective in changing melatonin release from the pineal gland. The concomitant changes of pineal melatonin release and extracellular pineal noradrenaline levels provided clear evidence for the involvement of the sympathetic innervation of the pineal gland in these PVN-induced changes. In a number of subsequent experiments, we were able to show that the GABAergic neurons in the SCN mediate the direct inhibitory effects of light on melatonin release and that they are involved in the circadian control of the melatonin rhythm. Additional experiments indicated that, in addition to the inhibitory control by GABA, the SCN lesion also removed an important stimulatory input to the melatonin rhythm-generating system (Perreau-Lenz et al., 2004). Consequently, the nocturnal peak of melatonin release implies that to provide this stimulatory input, SCN neurons should be active during the dark period. In fact, already a few years after the
discovery of the SCN, Zatz and Brownstein (1979) hinted upon this conclusion. However, this nocturnal activity is in apparent contrast with the suggested nocturnal silence of SCN neurons, indicated by the electrophysiological multiunit recordings and 2-deoxyglucose studies (Schwartz and Gainer, 1977; Groos and Hendriks, 1982). On the other hand, the immediate decrease of pineal melatonin release upon bilateral administration of TTX in the SCN clearly proved the necessity of nocturnal activity of SCN neurons (Perreau-Lenz et al., 2004). Therefore, although the nocturnal (electrical) activity of SCN neurons may seem rather weak, it is sufficient, and even necessary, to stimulate nocturnal melatonin release (i.e., the SCN-lesion studies mentioned before also showed that melatonin release is not due to the mere release from inhibition). The nocturnal electrical activity of SCN neurons probably remained unnoticed for such a long time because it is restricted to only a limited number of (glutamatergic) neurons.

Of course, the idea just described, that is, preautonomic hypothalamic neurons are controlled by a balance of glutamatergic and GABAergic inputs, could very well also apply to other rhythms under autonomic control. Indeed, a similar control mechanism seems to exist for the daily rhythm of plasma glucose concentrations (Fig. 3). In this case, it is the activity of preautonomic PVN neurons connected to the sympathetic innervation of the liver, which, during the light period, is restrained by a GABAergic inhibition derived from the SCN. In addition, the final activity of these preautonomic neurons, dedicated to the control of hepatic glucose production, seems to be determined by a balance of GABAergic and glutamatergic inputs (Kalsbeek et al., 2004). The daily rhythms in body temperature and cardiovascular activity are under SCN control as well and are clearly dependent on the autonomic nervous system (Scheer et al., 2003). In addition, the sleep/wake regulatory system in the VLPO seems to depend on the balance of GABA/glutamate inputs from the SCN as well (Sun et al., 2001).

SCN OUTPUTS AND THE BALANCE OF LIFE

The autonomic nervous system commands the organs through 2 antagonistic branches: the sympathetic nervous system, predominant in the active period ("fight, fright, flight"), and the parasympathetic nervous system, which rules the body during the inactive period ("rest and digest"). The cardiovascular system and pancreatic insulin release are the 2 clearest examples of physiological systems controlled via the parasympathetic branch of the autonomic nervous system. The few studies performed so far all indicate that a circadian rhythm in vagal cardiac tone is the main cause for the circadian rhythm in resting heart rate (Scheer et al., 2003). In addition, the finding of a pronounced daily modulation of the feeding-induced insulin response supports the existence of a circadian modulation of parasympathetic activity (Kalsbeek and Strubbe, 1998). However, at present it is not known whether the GABA/glutamate control mechanism, as described for the sympathetic branch of the autonomic nervous system, also applies to an SCN control of the parasympathetic branch of the autonomic nervous system.

Tracing studies on the white adipose tissue (WAT) innervation showed different neurons in the sympathetic ganglia to be in control of inguinal and epididymal fat pads, and different sympathetic and parasympathetic neurons within the central nervous system to be in control of subcutaneous and abdominal WAT compartments (Shi and Bartness, 2001; Kreier et al., 2002). Recently, we showed that this differentiation persists up to the hypothalamic preautonomic neurons and even the SCN (Kreier et al., 2006). To determine to what extent the biological clock can selectively affect parasympathetic and sympathetic autonomic outputs, we used virus tracing (with differently labeled viruses) combined with selective denervations. This approach revealed a complete separation of presympathetic and preparasypathetic neurons in the PVN (Fig. 4). Moreover, this segregation persisted up into the SCN. This compartmentalization of autonomic motor neurons provides the neuroanatomical basis for selective changes of the sympatho-parasympathetic balance in different compartments of the body and has resulted in hypotheses about the involvement of an unbalanced and arrhythmic autonomic nervous system in, for instance, the etiology of the metabolic syndrome (Hastings et al., 2003; Kreier et al., 2003). It is to be expected that a reduced activity of the biological clock or a misalignment of the endogenous biological clock rhythm with the exogenous environment will result in an unbalanced output toward the autonomic nervous system. In our opinion, it is this unbalanced activity of the autonomic nervous system that connects a long-term impaired functioning of the SCN to the genesis of the diseases of modern society,
Figure 3. Basal plasma glucose, insulin, and glucagon concentrations across the 24-h light/dark cycle in intact rats under conditions of ad libitum feeding (left panels) or a scheduled feeding regimen of 6 meals a day (i.e., 1 meal every 4 h) for several weeks (right panels). The scheduled feeding regimen has clear effects on the release pattern of the pancreatic hormones insulin and glucagon, but it hardly affects the daily plasma glucose rhythm. In view of the lack of a good hormonal candidate that could mediate the control of the central biological clock on hepatic glucose production, we started to explore a possible role for the autonomic nervous system. Horizontal dotted lines in the left-side panels indicate the mean glucagon and insulin levels during the light period (i.e., upper lines are mean ± SEM [standard error of mean], and lower lines indicate the mean – SEM). Vertical dotted lines in the right-side panels indicate the timing of the 10-min meals every 4 h. Adapted from La Fleur et al. (1999) and Ruiter et al. (2003).
such as obesity, type 2 diabetes, and hypertension. Indeed, postmortem studies have suggested a profound reduction of SCN activity in hypertensive patients (Goncharuk et al., 2002). Whether this underlies, in part, the beneficial effect of prolonged nighttime melatonin administration in hypertensive patients on blood pressure and sleep remains to be seen (Scheer et al., 2004). A number of clock gene knockout mice show metabolic disturbances (Rudic et al., 2004; Turek et al., 2005), but at present, it is not clear whether these deficits are a direct consequence of the malfunctioning clockwork or the indirect result of the loss of other (noncircadian) functions of the clock genes. However, the most convincing evidence to support the above hypothesis is the increased prevalence of obesity and the metabolic syndrome in shift workers, aging, and short sleep duration (Foster and Wulff, 2005).

HETEROGENEITY OF SCN OUTPUTS

The multifactorial control of the corticosterone and melatonin rhythm clearly shows that the temporal profile of an overt rhythm may differ substantially from that of the circadian output signal that regulates it. Moreover, in “real life,” the timing and waveforms of manifest rhythms are often shaped by circadian as well as noncircadian factors. Nevertheless, our physiological data indicate the existence of at least 4 subpopulations of SCN neurons responsible for the control of SCN output that have a differential timing of their acrophase in (electrical) activity. The 1st population (acrophase at ~ZT 2) contains VP and GABA and inhibits the release of corticosterone and hepatic glucose production, respectively. The 2nd and probably main population (acrophase at ~ZT 6) contains GABA, at least for some part, and is responsible for the inhibition of melatonin release during the light period. The 3rd population (acrophase at ~ZT 10) expresses an as yet unknown transmitter and is responsible for the activation of the HPA axis during its daily peak. Finally, neurons of the 4th population are mainly active during the subjective dark period (acrophase at ~ZT 18), at least some of them contain glutamate, and they are responsible for the nocturnal peak in melatonin release. Remarkably, a similar distinction of discrete phase groups of SCN neurons has recently also been defined on the basis of molecular and electrophysiological recordings. Schaap et al. (2003) showed that subpopulations of SCN neurons have surprisingly short periods (~5 h) of enhanced electrical activity, with peak activities occurring at different phases of the circadian cycle. Together, the activity of these subpopulations accounted for the neuronal ensemble pattern of SCN activity. At about the same time, Quintero et al. (2003) presented their results on the time-lapse imaging of SCN slices from mice with a short–half-life, green, fluorescent protein reporter of the \textit{Per1} gene. They discerned 4 groups of cellular oscillators, with the majority of neurons (50%-60%) forming a main phase group at ~CT 6, and 2 additional groups (10%-20%) peaking 3 to 4 h earlier or later. In addition, a less numerous group of neurons cycled with peak times primarily in the dark phase.

Moreover, the differential timing in the acrophases of hepatic glucose production and pineal melatonin release implicates a different timing of the trough in GABA release from SCN terminals that target the preautonomic neurons controlling the sympathetic input to the liver versus the ones controlling the sympathetic input to the pineal. Therefore, these data indicate that even within neurons with the same phenotype (i.e., GABAergic), subpopulations with a differential timing may exist. The presence of GABAergic neurons in the SCN colocalized with

**Figure 4.** Schematic view of the autonomic interactions between the hypothalamic suprachiasmatic (SCN) and paraventricular (PVN) nuclei, and peripheral organs. Separate sympathetic or parasympathetic neurons of the SCN project to preautonomic neurons of the PVN, where a similar sympathetic-parasympathetic separation can be observed. Preautonomic neurons of the PVN project either to the preganglionic sympathetic neurons in the intermediolateral column of the spinal cord (IML) or to the preganglionic neurons of the dorsal motor nucleus of the vagus (DMV). Moreover, the presynaptic PVN neurons have collaterals to preparasympathetic neurons, either in the PVN itself or to the nucleus tractus solitarius (NTS). Sympathetic and parasympathetic connections are represented by continuous and dashed lines, respectively. Adapted from Buijs et al. (2003).
either VP, VIP, or somatostatin also supports this possibility (Buijs et al., 1995).

SCN OUTPUTS AND PERIPHERAL CLOCKS

The SCN is crucial for the generation of daily rhythms throughout the mammalian body, but the (rhythmic) expression of clock genes is not unique to the SCN. The discovery of rhythmic clock gene expression throughout the entire body has led to a reconsideration of the relation between the master oscillator in the SCN and peripheral rhythms. Even the status of the SCN as master oscillator has come under scrutiny. However, viable transplants of non-SCN cell types do not restore behavioral rhythms in SCN-lesioned animals (Earnest et al., 1999), and coculture models showed that only SCN cells, but not fibroblasts, can confer molecular and metabolic rhythms to co-cultured cells (Jin et al., 1999). According to the current opinion, the central pacemaker in the SCN coordinates the activity of local oscillators in the peripheral tissues via behavioral, neuroendocrine, and autonomic pathways (Buijs and Kalsbeek, 2001; Terazono et al., 2003; Guo et al., 2005). However, a clear understanding of the role of peripheral oscillators in the regulation of the physiological functions of peripheral organs is still lacking. Previous experiments showed that both daily rhythms in liver clock gene expression and plasma glucose concentrations are maintained during fasting (La Fleur et al., 1999; Kita et al., 2002). Moreover, both hepatic PER expression and hepatic glucose production are increased by the activation of the sympathetic input to the liver (Terazono et al., 2003; Kalsbeek et al., 2004). Therefore, we investigated whether peripheral oscillators in the liver are a necessary link in the transfer of the circadian information from the biological clock to hepatic glucose production. In line with the hypothesis proposed in our previous denervation study, removal of the sympathetic input to the liver resulted in an obliteration of the daily rhythm in plasma glucose levels. Contrary to our expectations, however, transcript levels of the 5 clock genes studied maintained their rhythmicity in the liver (Cailotto et al., 2005). Thus, these results clearly show that 1) autonomic innervation of the liver is not required to sustain the rhythmicity of peripheral oscillators in the liver and 2) rhythmic expression of the peripheral oscillators in the liver is not sufficient to maintain a rhythmic output of the liver (i.e., glucose production). Thus, these experiments provide no evidence for a functional significance of peripheral oscillators in the rhythmic output of a tissue.

SCN OUTPUTS AND DIURNALITY

Most medical and chronobiological research is performed in nocturnal mammals, and consequently, knowledge on the SCN output mechanisms in diurnal species is virtually nonexistent. Due to this paucity of functional insight, a large amount of data collected on human SCN morphology and transmitter content associated with observed pathology in life is difficult to interpret. Therefore, it is important to identify the neural processes underlying the differences between nocturnal and diurnal mammals so that research findings from nocturnal species are not incorrectly assumed to apply to diurnal humans (i.e., the investigation of the sleep-promoting effect of melatonin in rats). Relative to the L/D cycle, the circadian rhythms of neural, metabolic, neurotransmitter, and clock gene expression in SCN neurons are similarly phased in nocturnal and diurnal animals (Smale et al., 2003), the diurnal chronotype apparently being generated downstream of the SCN. The efferent projections of the “diurnal SCN” do not show obvious differences with those of the “nocturnal SCN” (Novak et al., 2000), and overall the gross anatomic substructures and major neuronal connections of the SCN are also conserved in humans, implying conservation of the neuroendocrine and autonomic control of rhythms by the SCN across chronotypes (Dai et al., 1998). Moreover, some species are even able to switch between a nocturnal and diurnal habitat, depending on seasonal or environmental conditions (Mrosovsky and Hattar, 2005). Therefore, the nocturnal/diurnal difference does not seem to reside in the hardware (i.e., wiring) of the SCN, but more likely in the composition of its output (i.e., an exchange of stimulatory and inhibitory outputs) or in the translation of the SCN output in its target areas (i.e., inhibitory or stimulatory interneurons). Indeed, as indicated before, the major amount of SCN projections is directed toward target areas that contain mainly interneurons (Saper et al., 2005), rather than directly toward endocrine or preautonomic “motorneurons” (Kalsbeek and Buijs, 2002). On the other hand, the sympathetic output to the pineal is stimulated during the dark period in both nocturnal and diurnal species, but the timing of the stimulatory input of the autonomic nervous system
to other physiological functions, for example, body temperature and cardiovascular activity, is often linked to the activity state of the animal. Furthermore, nighttime exposure to environmental light—as a signal of the day—has an opposite influence on temperature, corticosteroid release, and cardiovascular regulation in humans compared to rats (Buijs et al., 1999; Scheer et al., 2003). It is hard to imagine that these different effects are only due to masking (Mrosovsky and Hattar, 2005). This means that during the light period, and possibly during nocturnal light exposure as well, GABAergic projections from the SCN inhibit “pineal” and “cardiovascular” PVN neurons simultaneously in nocturnal species, whereas in diurnal species, the pineal and cardiovascular PVN neurons need to be inhibited separately during the light and dark period. This not only indicates that the SCN should be able to target pineal and cardiovascular PVN neurons separately but also raises the question how the SCN can change the nature of “cardiovascular” PVN neurons from nocturnal to diurnal.

Indeed, we recently obtained anatomical evidence that such a specialization might exist in the SCN (Kreier et al., 2006). Thus far, only the SCN control of the daily surge in luteinizing hormone (LH) has been compared in some detail between a nocturnal and a diurnal species, the laboratory rat and the unstriped Nile grass rat (Arvicanthis niloticus), respectively. The 12-h difference in the timing of the LH surge in these 2 species is in all likelihood due to differences in GnRH neuron activation, as the rise in c-fos expression in these neurons also occurs 12 h apart (Van Der Beek et al., 1994; Mahoney et al., 2004). In both species, GnRH- and ER-containing neurons are contacted by VP- and VIP-containing SCN fibers (De La Iglesia et al., 1995; Van Der Beek et al., 1997; Mahoney and Smale, 2005). However, because localized infusions of SCN transmitters have thus far only been performed in rats (Palm et al., 1999), it is still unclear how the 12-h difference in GnRH activation between the 2 species is accomplished by the SCN output.

CONCLUSION

From the above, it will be clear that, in mammals, the output of the hypothalamic biological clock not only controls the daily rhythm in sleep/wake (or feeding/fasting) behavior but also exerts direct control over many physiological and neuroendocrine rhythms. The balanced “ying-yang” relationship of repressing and activating elements that is so clearly present in the molecular machinery of the circadian clock seems to be a characteristic element of the circadian system that can be recognized at different levels of the circadian control system, from the behavioral sleep/wake system to the level of single hormone systems. With a lesser or completely vanishing control of the biological clock, the remaining level of behavior or hormone release will depend on the previous ratio of inhibitory and stimulatory SCN inputs. The remaining behavior or hormone release can be set either at the mean of the previous day/night rhythm (sleep/wake behavior, corticosterone release, plasma glucose concentrations) or in the direction of the previous peak (leptin release, glucose uptake) or trough levels (melatonin release, insulin sensitivity).

In our opinion, one of the most important aspects of the output of the biological clock is its control of the sympathetic/parasympathetic balance in autonomic nervous activity. In view of their antagonistic properties and the widespread influence of the autonomic nervous system on the physiological state of an organism, the control of this autonomic balance also controls the daily balance of our lives. Therefore, a well-entrained biological clock is essential for a balanced autonomic nervous system and may have protective value as far as diseases characterized by an imbalance in the autonomic nervous system are concerned, such as hypertension, type 2 diabetes, and the metabolic syndrome.

APPENDIX

ABBREVIATIONS

<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<tr>
<td>CRH</td>
<td>Corticotrophin releasing hormone</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>CT</td>
<td>Circadian time</td>
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<td>DMH</td>
<td>Dorsomedial nucleus of the hypothalamus</td>
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<td>DMV</td>
<td>Dorsal motor nucleus of the vagus</td>
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<tr>
<td>ER</td>
<td>Estrogen receptor</td>
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<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
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<td>GRP</td>
<td>Gastrin releasing peptide</td>
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<td>GnRH</td>
<td>Gonadotrophin releasing hormone</td>
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<td>HPA</td>
<td>Hypothalamo-pituitary-adrenal</td>
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<td>HPG</td>
<td>Hypothalamo-pituitary-gonad</td>
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<td>HPT</td>
<td>Hypothalamo-pituitary-thyroid</td>
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<td>L/D</td>
<td>Light/Dark</td>
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<td>LH</td>
<td>Luteinizing hormone</td>
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<td>LHRH</td>
<td>Luteinizing hormone releasing hormone</td>
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<td>MPOA</td>
<td>Medial preoptic area</td>
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<td>NTS</td>
<td>Nucleus tractus solitarius</td>
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